Scientific Opinion supplementing the conclusions of the environmental risk assessment and risk management recommendations for the cultivation of the genetically modified insect resistant maize Bt11 and MON 810

Arpaia, Salvatore ; Birch, Nicholas ; Chesson, Andrew ; du Jardin, Patrick ; Gathmann, Achim ; Gropp, Jürgen ; Herman, Lieve ; Hoen-Sorteberg, Hilde-Gunn ; Jones, Huw ; Kiss, Jozsef ; Kleter, Gijs ; Lovik, Martinus ; Messéan, Antoine ; Naegeli, Hanspeter ; Nielsen, Kaare M ; Ovesna, Jaroslava ; Perry, Joe ; Rostoks, Nils ; Tebbe, Christoph

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SCIENTIFIC OPINION

Scientific Opinion supplementing the conclusions of the environmental risk assessment and risk management recommendations for the cultivation of the genetically modified insect resistant maize Bt11 and MON 810[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The EFSA GMO Panel was asked by the European Commission to apply its mathematical model to simulate and assess potential adverse effects resulting from the exposure of non-target Lepidoptera to maize Bt11 or MON 810 pollen under hypothetical agricultural conditions, and to provide information on the factors affecting the insect resistance management plan, additional to that in its 2011 Statement supplementing the evaluation of the environmental risk assessment and risk management recommendations on insect resistant genetically modified maize Bt11 for cultivation. Here, risk managers are provided with additional evidence and further clarifications to those previous conclusions and risk management recommendations. This Scientific Opinion provides background scientific information to inform the decision-making process; the EFSA GMO Panel reiterates that risk managers should choose risk mitigation and management measures that are proportionate to the level of identified risk according to the protection goals pertaining to their regions.

KEY WORDS

Bt11, Cry1Ab, environmental safety, GMO, insect resistance, maize (*Zea mays*), mathematical modelling, MON 810, non-target Lepidoptera

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[2] Panel members: Salvatore Arpaia, Nicholas Birch, Andrew Chesson, Patrick du Jardin, Achim Gathmann, Jürgen Gropp, Lieve Herman, Hilde-Gunn Hoen-Sorteberg, Huw Jones, Jozsef Kiss, Gijs Kleter, Martinus Lovik, Antoine Messéan, Hanspeter Naegeli, Kaare M. Nielsen, Jaroslava Ovesná, Joe Perry, Nils Rostoks, Christoph Tebbe. Correspondence: gmo@efsa.europa.eu
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SUMMARY

In its 2011 Statement supplementing the evaluation of the environmental risk assessment and risk management recommendations on genetically modified insect resistant maize Bt11 for cultivation, the EFSA GMO Panel used the mathematical model, initially developed for maize MON 810 and later recalibrated for maize 1507, to simulate and assess potential adverse effects resulting from the exposure of non-target (NT) Lepidoptera (butterflies and moths) to pollen from maize Bt11 under representative EU cultivation conditions. The model was also used to estimate the efficacy of certain mitigation measures. The EFSA GMO Panel concluded that risk mitigation measures may be needed under specific conditions (depending on e.g., sensitivity and occurrence of NT Lepidoptera, acreage of *Bt*-maize, host-plant density) in order to reduce the exposure of extremely sensitive NT Lepidoptera to maize Bt11 pollen. The EFSA GMO Panel considered that the conclusions on the risk to NT Lepidoptera from maize Bt11 apply equally to maize MON 810. The EFSA GMO Panel also reiterated its recommendation that appropriate insect resistance management (IRM) strategies relying on the 'high dose/refuge' strategy should be employed, in order to delay the potential evolution of resistance to the Cry1Ab protein in lepidopteran target pests.

In this Scientific Opinion, the EFSA GMO Panel was asked by the European Commission to re-apply its mathematical model to consider additional hypothetical agricultural conditions, and to provide more information on the factors affecting the IRM plan, in order to supplement its 2011 Statement on maize Bt11. Here, risk managers are provided with additional evidence and further clarifications to those previous conclusions and risk management recommendations.

Depending on the level of exposure to *Bt*-maize pollen, there is a potential hazard to NT lepidopteran larvae on their host-plants in fields cropped with non-Lepidoptera-active crops when they neighbour the maize Bt11/MON 810 field under consideration. However, the need for risk management should consider the distance from the nearest source of *Bt*-maize pollen and hence their exposure as well as the pest status of the species concerned.

Within agricultural landscapes, when a field cropped with maize Bt11/MON 810 has no margins containing host-plants of NT lepidopteran larvae, the only larvae exposed are those on any host-plants occurring within the GM crop. When such host-plants are present, a greater percentage of the larvae exposed to *Bt*-maize pollen are expected to suffer mortality than when a field has margins with host-plants.

If a maize Bt11/MON 810 field has margins, then sown strips of non-*Bt*-maize, placed between the edges of the *Bt*-maize crop and each margin, are considerably more effective as a mitigation measure at reducing expected mortality than a single block of non-*Bt*-maize of comparable area, wherever the latter is planted. This is the case whether there are host-plants in the crop or not. By contrast, when a maize Bt11/MON 810 field has no margins, then a single block of non-*Bt*-maize is slightly more effective than sown strips at reducing mortality of NT lepidopteran larvae.

For NT lepidopteran species of conservation concern occurring within protected habitats, it is appropriate for thresholds used to derive recommendations for risk management to be based on a criterion of local mortality; for NT lepidopteran larvae occurring within maize fields and their margins a criterion of global mortality is considered appropriate.

Seed mixtures (e.g., *refugia* in a bag) provide the poorest possible efficacy of mitigation because they do little to limit the exposure of non-target Lepidoptera to *Bt*-maize pollen.

In general, for any particular *Bt*-maize plant expressing Cry1 protein, the required isolation distance around protected habitats within which sources of maize Bt11/MON 810 pollen should not be cultivated increases with: (a) the sensitivity of the NT lepidopteran larvae and (b) the expression levels of the Cry1-protein in *Bt*-maize pollen. In the present case of maize Bt11/MON 810, it is confirmed that imposing an isolation distance of 20 m around a protected habitat from the nearest crop of maize

Bt11/MON 810 would be expected to reduce local mortality, even of extremely highly sensitive non-target lepidopteran larvae, to a level at or below 0.5%. This estimated isolation distance is conservative, since it assumes extremely high levels of sensitivity in NT lepidopteran larvae, and because larvae within the habitat will be at greater distances from the *Bt*-maize crop than those on the edge of the habitat.

The EFSA GMO Panel considers that the potential of regionally occurring non-target lepidopteran pests to evolve resistance exists but is considerably less than that of target pests and therefore routine IRM would not be proportionate. Hence, the EFSA GMO Panel reiterates that regionally occurring non-target lepidopteran pests should be considered within the PMEM. General surveillance shall be used to report information on unexpected larval damage to maize and observations on the occurrence and survival of lepidopteran larvae on *Bt*-maize plants. In addition, monitoring reports from regional plant inspection services should be used to trigger subsequent investigations, including CSM if necessary. The applicants should amend their PMEM plans accordingly.

This Scientific Opinion provides background scientific information to inform the decision-making processes; the EFSA GMO Panel reiterates that risk managers should choose risk mitigation and management measures that are proportionate to the level of identified risk according to the protection goals pertaining to their regions.

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BACKGROUND AS PROVIDED BY EFSA

On 30 November 2011, the EFSA GMO Panel issued a Statement supplementing the evaluation of the environmental risk assessment and risk management recommendations on genetically modified (GM) insect resistant maize Bt11 for cultivation (EFSA, 2011a). In that Statement, the EFSA GMO Panel further analysed the potential adverse effects of maize Bt11 pollen on NT Lepidoptera and, in this respect, further clarified its previous recommendations to risk managers (EFSA, 2005). In EFSA (2011a), the EFSA GMO Panel concluded: "*that locally exposed non-target Lepidoptera that are 'extremely sensitive' to the Cry1Ab protein may be at risk if exposed to harmful amounts of maize Bt11 pollen*". Therefore, the EFSA GMO Panel concluded that risk mitigation measures (e.g., non-*Bt*-maize strips between the edges of maize Bt11 field and the field margins) are only required in situations where 'extremely sensitive' NT Lepidoptera populations might be at risk. For example, this is likely when 'extremely highly sensitive' NT Lepidoptera and their host-plants were present in *Bt*-maize fields and margins in areas where there is a high proportion of maize in arable fields and a high rate of adoption of maize Bt11 (and/or other Lepidoptera-active maize events such as maize MON 810 currently grown in the EU). In the habitats as defined according to Directive 2004/35/EC (EC, 2004) where protected Lepidoptera species are present, the EFSA GMO Panel considers that a distance of 20 m is sufficient to reduce the mortality to a negligible level below 0.2% in the margins of the protected areas, even for 'extremely highly sensitive' species (EFSA, 2011a).

Furthermore, in light of the similarities between both Cry1Ab-expressing maize transformation events Bt11 and MON 810 (e.g., identity of amino acid sequence of the core protein, similar biological activity against sensitive Lepidoptera, similar Cry1Ab protein expression level in pollen), the EFSA GMO Panel considered that the conclusions on the risk to NT Lepidoptera from maize Bt11 apply equally to maize MON 810 (EFSA, 2011a).

The EFSA GMO Panel also reiterated its recommendation that appropriate IRM strategies relying on the 'high dose/refuge' strategy should be employed, in order to delay the potential evolution of resistance to the Cry1Ab protein in lepidopteran target pests. In the case of a cluster of fields with an aggregate area greater than 5 ha of *Bt*-maize, the EFSA GMO Panel advises that there shall be non-*Bt*-*refugia* equivalent to 20% of this aggregate area, irrespective of individual field and farm size (EFSA, 2009, 2011a). For maize Bt11, the EFSA GMO Panel also recommended the applicant to consider the need for IRM and CSM to account for the presence of other Cry1Ab-expressing maize events such as maize MON 810, currently grown in the EU (EFSA, 2011a). Specifically, where more than one Cry1Ab-expressing maize event is grown on a farm, then a single IRM plan should be adopted so that all of the areas of *Bt*-maize can be considered together for the purposes of defining *refugia*.

In this Scientific Opinion, the EFSA GMO Panel was asked to re-apply the mathematical model to consider additional hypothetical agricultural conditions and to provide more information on the factors affecting the IRM plan previously assessed by the EFSA GMO Panel in its 2011 Statement on maize Bt11[4].

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION AND EFSA

The European Commission requested EFSA to provide additional evidence and to further clarify certain elements of the 2011 EFSA GMO Panel Statement supplementing the evaluation of the environmental risk assessment and risk management recommendations on GM insect resistant maize Bt11 for cultivation (EFSA, 2011a). In particular, the European Commission requested the EFSA GMO Panel to answer the following four questions by applying the mathematical model proposed by Perry et al. (2011, 2012) to additional agricultural hypothetical conditions:

(1) To calculate the local mortality of NT Lepidoptera where there is a strip of non-*Bt*-maize that is 21 m wide around the perimeter of the *Bt* crop and either a) 2 m field margin or b) no field margins;

[4] In EFSA (2011a), the EFSA GMO Panel considers that: *"the conclusions on the risk to non-target Lepidoptera from maize Bt11 apply equally to maize MON 810"*.

(2) To consider the influence of non-*Bt-refugia* spatial arrangements on the local mortality of NT Lepidoptera;

(3) To calculate the local mortality of NT lepidopteran species with increasing distances from the nearest maize Bt11/MON 810 field and where there are no field margins;

(4) To consider the influence of local and regional conditions on IRM plans.

ASSESSMENT

1. INTRODUCTION

Both maize transformation events, Bt11 and MON 810, were developed to express a Cry1Ab protein variant, derived from *Bacillus thuringiensis* subsp. *kurstaki*, which confers protection against the lepidopteran target pests European corn borer (ECB, *Ostrinia nubilalis* Hübner) and Mediterranean corn borer (MCB, *Sesamia nonagrioides* Lefebvre). Maize Bt11 also expresses the phosphinothricin-N-acetyltransferase (PAT) protein from *Streptomyces viridochromogenes*, which confers tolerance to the herbicidal active substance glufosinate-ammonium. The PAT protein expressed in maize Bt11 has been used as selectable marker to facilitate the selection process of transformed plant cells and is not intended for weed management purposes.

2. SUMMARY OF PREVIOUS CONCLUSIONS AND RECOMMENDATIONS BY THE EFSA GMO PANEL

The potential of maize Bt11 and MON 810 to have adverse effects on non-target organisms (NTOs) and the ecosystem services they provide in agro-ecosystems was previously evaluated by the EFSA GMO Panel (EFSA, 2005, 2006, 2008, 2009) and the outcome of these evaluations has been recently updated in the light of new relevant scientific literature and advances in methodology (EFSA, 2011a, 2012a,b).

Using a mathematical model of exposure to assess potential adverse effects resulting from exposure of NT lepidopteran species to Cry1Ab-containing maize pollen deposited on their host-plants under representative cultivation conditions (Perry, 2011a; Perry et al., 2010, 2011, 2012 referred to in EFSA, 2011a), the EFSA GMO Panel concluded "*that locally exposed non-target Lepidoptera that are 'extremely sensitive' to the Cry1Ab protein may be at risk if exposed to harmful amounts of maize Bt11 pollen*" (EFSA, 2011a). The EFSA GMO Panel proposed to risk managers the implementation of mitigation measures only in situations[5] where 'extremely sensitive' NT Lepidoptera populations within maize arable agro-ecosystem (i.e., field cropped with maize Bt11 and its margins) might be at risk. In EFSA (2011a), the EFSA GMO Panel considered that: *"the conclusions on the risk to non-target Lepidoptera from maize Bt11 apply equally to maize MON 810"*.

Here, and in EFSA (2011a,b, 2012c), the term 'margin' follows the definition adopted in Figure 1 of Roy et al. (2003), and includes the three components of the uncropped land at the edge of a field: any tilled strip, any verge of herbaceous or grassy vegetation, and the living or non-living field boundary. Where the term 'no margin' is used, it means a field with either a non-living boundary, or with uncropped land at its edge that has no host-plants of the NT lepidopteran species under consideration. The mitigation measures recommended were the planting of border rows of non-Lepidoptera-active maize (hereafter abbreviated as non-*Bt*-maize) at the edges of the maize Bt11/MON 810 crop, between the crop and any component of the margin. This could: (1) reduce the exposure to maize Bt11/MON 810 pollen to any lepidopteran individuals feeding on host-plants occurring within the margins and (2) contribute to the required percentage of non-*Bt*-maize necessary to constitute non-*Bt*-*refugia* for lepidopteran target pests in the framework of IRM (EFSA, 2009, 2011a).

Here, and in EFSA (2011a,b, 2012c), mortality is estimated in two phases: firstly locally, using the 'small-scale' parameters, and then globally, using the 'large-scale' parameters. The term 'locally' means spatially within the crop and its immediate margins, and temporally within the period of pollen shed. The term 'globally' means after averaging over an entire landscape or regional scale and over a whole growing season. The EFSA GMO Panel focuses on providing estimates of mortality at the local, small-scale and giving information that will enable risk managers to translate these to global estimates of mortality appropriate to the region modelled.

[5] *For example, when 'extremely sensitive' non-target Lepidoptera and their host-plants are present in Bt-maize fields and margins in areas where there is a high proportion of maize in arable fields and a high rate of adoption of maize Bt11 (and/or other Lepidoptera-resistant maize events such as maize MON 810 currently grown in the EU)* (EFSA, 2011a).

In EFSA (2011a), the EFSA GMO Panel indicated that: *"Special attention should be paid to the degree of large-scale exposure as risk mitigation measures are only needed when the proportion and uptake of maize Bt11 (and/or other Lepidoptera-resistant maize events such as maize MON 810 currently grown in the EU) are sufficiently high, regardless of the other parameters. If maize Bt11 (and/or maize MON 810) cultivation remains below 7.5% of the regional Utilized Agricultural Area[6,7], the global mortality is predicted to remain below 1%, even for 'extremely sensitive' species, and then risk mitigation measures using non-Bt-maize border rows are not required"*. Regarding the local mortality of NT lepidopteran species of conservation concern in protected habitats according to Directive 2004/35/EC (EC, 2004), the EFSA GMO Panel considered that: *"a distance of 20 m is sufficient to reduce the mortality to a negligible level below 0.2% in the margins of the protected areas, even for 'extremely sensitive' species"* (EFSA, 2011a).

Furthermore, the EFSA GMO Panel also reiterated its recommendation that appropriate IRM strategies relying on the 'high dose/refuge' strategy should be employed, in order to delay the potential evolution of resistance to the Cry1Ab protein in lepidopteran target pests. In the case of a cluster of fields with an aggregate area greater than 5 ha of *Bt*-maize, the EFSA GMO Panel advises that there shall be non-*Bt-refugia*[8] equivalent to 20% of this aggregate area, irrespective of individual field and farm size (EFSA, 2009, 2011a). The EFSA GMO Panel also recommended the applicant to consider integrating the IRM and CSM for maize Bt11 with that of other Cry1Ab-expressing maize events such as maize MON 810 currently grown in the EU (EFSA, 2011a).

3. ENVIRONMENTAL RISK ASSESSMENT

3.1. General additional information on the mortality of non-target Lepidoptera

3.1.1. Presence of neighbouring crops as host-plants

It is possible that neighbouring crops (e.g., oilseed rape) to a *Bt*-maize field could be considered as host-plants of larvae of certain species of NT lepidopteran. In these crops, NT lepidopteran larvae could be exposed to *Bt*-maize pollen especially in situations where there are little or no field margins containing non-crop plants. This possibility was considered by Perry et al. (2012) in their Appendix S4, where it was stated: "*for example, larvae of the monovoltine moth Noctua tirrenica feed on Brassicaceae, largely between September and October, and therefore suffer negligible overlap with pollen shed in maize in central Europe"*. Note the caveat in Appendix S4 of Perry et al. (2012), that points out that exposure depends on the temporal coincidence of larvae on these host-plants with maize anthesis and pollen shed. Another example given was *Phragmatobia fuliginosa*, but it was noted that this species is highly polyphagous and has a wide range of host-plants which include, but are by no means restricted to Brassicaceae and maize (Schmitz et al., 2003). The EFSA GMO Panel emphasizes that, when a NT lepidopteran species has several host-plant species, this would need to be accounted for in the exposure calculations, and those calculations would require considerable data regarding relative preferences of hosts and relative densities of all the host-plants, in order to estimate the risk.

In addition, it should be acknowledged that a species which has a host-plant that is a crop is often a pest of that crop. In EFSA (2011a,b, 2012c), the mortality of individuals of a pest species should not necessarily be considered as an environmental hazard *per se*. For example, *Plutella xylostella* is the most important pest of Brassicaceae in the world. Hence, whilst individual larvae of *P. xylostella*[9] might feed on an oilseed rape crop closely adjacent to a *Bt*-maize crop and could be adversely affected by pollen from the latter, this need not necessarily constitute an environmental hazard. Pest species, by

6 For example, a maximum uptake of 25% of maize Bt11 (and/or maize MON 810) in a region where maize represents 30% or less of the arable land.

7 I.e., zv = 0.075, and with conservative assumptions for the other parameters y=a=x=0.5, yielding R = 0.009375.

8 Non-*Bt-refugia* are defined as non-Lepidoptera-active maize *refugia*.

9 The EFSA GMO Panel considers that the description of *P. xylostella* as a 'non-target organism' in the introductory section of Perry et al. (2012) should have been qualified to avoid confusion.

definition, usually have large populations at peak density, so in general there may be little need to mitigate against their mortality for conservation purposes. Also, it should be stressed that the "*other lepidopteran pests than the European and Mediterranean corn borer*" mentioned in various sections of EFSA (2011a) refers only to pests of maize.

There is a potential hazard to NT lepidopteran larvae on their host-plants in fields cropped with non-Lepidoptera-active crops when they neighbour the maize Bt11/MON 810 field under consideration. However, the estimated risk should take into account the level of exposure because of the distance of such larvae from the nearest source of maize Bt11/MON 810 pollen, especially if the field cropped with maize Bt11/MON 810 and/or the neighbouring field under consideration has a margin. The risk to such larvae will always be less than for larvae located in the margin of the maize Bt11/MON 810 field directly between them and the Bt11/MON 810 crop.

3.1.2. The criteria used as thresholds for mortality to derive recommendations for mitigation measures

3.1.2.1. Brief description of the mathematical model for exposure

In order to facilitate the reading of the present Scientific Opinion, the underlying assumptions of the mathematical model of exposure as referred to in EFSA (2011a) on maize Bt11 are repeated below (see also EFSA, 2009, 2012c; Perry, 2011a,b; Perry et al., 2010, 2011, 2012 for further details).

The model quantifies the potential risk of mortality to the NT lepidopteran larvae from maize Bt11 (and/or maize MON 810) through the ingestion of harmful amounts of pollen deposited on their host-plants for a square typical European maize field of area C = 15 ha with a margin of width D = 2 m (see definition of margin in Section 2, above).

The full exposure assessment accounts for three types of parameters: (1) parameters concerned with mortality (considering five assumed levels[10] of sensitivity); (2) small-scale parameters (considering two assumed within-crop host-plant densities[11] and a range of nine levels of mitigation[12] in the form of sown strips of non-*Bt*-maize); and (3) five large-scale parameters[13].

A global estimate of mortality, appropriate to the region modelled, is obtained by multiplying the local estimate of mortality by the multiplicative product of the five large-scale parameters (i.e., by the parameter denoted as R).

3.1.2.2. Non-target Lepidoptera occurring within maize fields and their margins

In EFSA (2011a), the stated aim of the EFSA GMO Panel was to provide risk managers with tools to estimate global and, where needed local, mortality of exposed NT Lepidoptera, on a case-by-case basis, both before and after different risk mitigation measures are put in place, and for different host-plant densities. This enables risk managers to choose risk mitigation measures proportionate to the level of identified risk and to the protection goals pertaining to their region. Global mortality was used as a criterion for NT lepidopteran larvae occurring within maize Bt11/MON 810 fields and their

[10] Five assumed levels of sensitivity: below-average, above-average, high, very high and extremely high levels of sensitivity of the NT Lepidoptera to the Cry1Ab protein from maize Bt11/MON 810.

[11] Two assumed within-crop host-plant densities (parameter e): 0.00 and 0.01 plants/m^2.

[12] Nine levels of mitigation in the form of sown strips of non-*Bt*-maize of different width (parameter w): 0, 3, ..., 24 m between the main crop and the field margin.

[13] The five 'large-scale' parameters are :

- y, the proportion of the lepidopteran host-plant that is found within arable crops and in their margins (as opposed to other habitats);
- z, the proportion of arable fields that are cropped with maize (as opposed to other crops) in any year in the region;
- v, the proportion of all maize sown within the defined region that is cropped with maize Bt11/MON 810;
- x, the proportion of larvae that remains exposed, after allowance for a set of physical and behavioural effects that tend to reduce exposure;
- a, the proportion by which exposure is reduced owing to lack of temporal coincidence between the susceptible larval stage concerned and the period over which pollen from maize Bt11/MON 810 is shed.

margins because it was recognised that species occurring within one maize Bt11/MON 810 field and its margins might be considered as one population within a metapopulation in different maize fields and elsewhere in the arable maize ecosystem, and that such a metapopulation could be linked by dispersal between such fields, and that recovery or recolonisation of each population was possible *sensu* Sherratt and Jepson (1993).

The EFSA GMO Panel emphasizes that the criteria used as thresholds for mortality to derive recommendations for risk management should be used as examples only. In EFSA (2011a), it was suggested that, based on estimates of global percentage mortality, it was possible for risk management strategies to be determined for each specific case (i.e., each species × region combination) according to protection goals (for that species in that region). As in EFSA (2011a,b, 2012c), the GMO Panel makes no attempt to pre-empt decisions concerning the formulation of such strategies, because this is a task for regional risk managers. Any threshold applied must of necessity be arbitrary and should be subject to amendment according to protection goals of each Member State.

For NT lepidopteran species occurring within *Bt*-maize fields and their margins, EFSA (2011a) continued: "*Special attention should be paid to the degree of large-scale exposure as risk mitigation measures are only needed when the proportion and uptake of maize Bt11 (and/or other Lepidoptera-resistant maize events such as maize MON 810 currently grown in the EU) are sufficiently high, regardless of the other parameters. If maize Bt11 (and/or maize MON 810) cultivation remains below 7.5% of the regional Utilized Agricultural Area*[14,15]*, the global mortality is predicted to remain below 1%, even for 'extremely sensitive' species, and then risk mitigation measures using non-Bt-maize border rows are not required… The EFSA GMO Panel concludes that risk mitigation measures are only required in situations where 'extremely sensitive' non-target Lepidoptera populations might be present and subject to sufficiently high exposure; for example, when 'extremely sensitive' non-target Lepidoptera and their host plants are present in Bt-maize fields and margins in areas where there is a high proportion of maize in arable fields and a high rate of adoption of maize Bt11 (and/or maize MON 810)*".

The recommendations for risk management used the same criterion for a threshold as an example in both EFSA (2011a) and EFSA (2011b); namely that if estimated global mortality exceeded 1%, then mitigation was recommended.

Conclusions were drawn by first assuming a value for the parameter R of 0.02 (i.e., a precautionary value used by Perry et al. (2010) greater than the value considered 'typical'), and then identifying the lowest category of lepidopteran larval sensitivity that gave an estimated global mortality of >1%. For example, the 2011 Scientific Opinion on maize Bt11 recommended given this criterion that: "*risk mitigation measures are only required in situations where extremely sensitive non-target Lepidoptera might be at risk*" (EFSA, 2011a). Note that, using the same criterion, the 2011 Scientific Opinion[16] on maize 1507 recommended that: "*risk mitigation measures are only required in situations where highly sensitive non-target Lepidoptera populations might be at risk*".

The transformation events, maize 1507 and maize Bt11 (and by analogy maize MON 810), have different toxicities, due to the different Cry1-protein expression levels in pollen and the different sensitivities of NT lepidopteran species to Cry1F and Cry1Ab proteins, respectively. Since the toxicity of the Cry1F protein from maize 1507 is generally greater for species studied to date than that of Cry1Ab protein from maize Bt11/MON 810, it is understandable that mitigation for maize 1507 is recommended for a wider range of NT lepidopteran species than for maize Bt11/MON 810 (EFSA, 2011a,b; 2012c).

[14] For example, a maximum uptake of 25% of maize Bt11 (and/or maize MON 810) in a region where maize represents 30% or less of the arable land.
[15] I.e., zv = 0.075, and with conservative assumptions for the other parameters y=a=x=0.5, yielding R = 0.009375.
[16] See Sections 2.3.5.2 (Figure X(d)) and 3.1.3 on risk mitigation measures in EFSA (2011b).

3.1.2.3. Non-target lepidopteran species of conservation concern occurring within protected habitats

In EFSA (2011a,b, 2012c), for NT lepidopteran species of conservation concern occurring at sites within protected habitats according to Directive 2004/35/EC (EC, 2004), the EFSA GMO Panel considered it more appropriate to use the criterion of local mortality, rather than global mortality, as a threshold to derive recommendations for risk management. This is for two reasons. Firstly, such sites in protected habitats are usually isolated, relatively small habitat patches containing specific food plants for Lepidoptera and which often lack nearby contiguous similar habitat from which colonisation or recovery would allow the replenishment of a population suffering decline through mortality (see Sherratt and Jepson, 1993). Secondly, populations of species of conservation concern often have relatively small populations which are usually not widespread; such populations are less able to tolerate mortality and may become locally extinct. Protection goals for such species would be expected to employ lower thresholds for mortality than for more common species occurring in maize fields and margins which may be widespread throughout the maize arable ecosystem.

Hence, in EFSA (2011a)[17], the EFSA GMO Panel made recommendations for mitigation measures, based on rounded isolation distances (rather than exact distances) from the nearest maize Bt11/MON 810 crop, that would be necessary to decrease the estimated local mortality below 0.5%, even for extremely highly sensitive NT lepidopteran species. The adoption of 0.5%, as an example, would achieve an estimated expected mortality rate of no more than one individual in every 200. It should be noted that such estimates are conservative, since the estimated mortality applies only to sensitive larvae at the outer margins of the protected habitat nearest to the *Bt*-maize crops; larvae within the habitat will be subject to a lower risk. Also, as above, since the toxicity of the Cry1F protein from maize 1507 is generally greater than that of Cry1Ab from maize Bt11/MON 810, it is understandable that the isolation distance estimated to reduce estimated local mortality to less than 0.5% for maize 1507 (i.e., 30 m) is greater than the corresponding distance for maize Bt11/MON 810 (i.e., 20 m, see EFSA (2011a)).

Furthermore, in EFSA (2011a), the EFSA GMO Panel considered that the proposal of an isolation distance of 20 m around such local protected habitats, within which maize Bt11/MON 810 should not be cultivated, would usually only reduce slightly the potential area within a region that could be planted with maize Bt11/MON 810. Given that, it seemed reasonable to recommend an isolation distance that would help protect all NT lepidopteran larvae, including those of extremely sensitive species, rather than a lower distance that would leave the larvae of some sensitive species exposed to an expected risk of greater than 0.5% local mortality for the example studied.

Again, notwithstanding these results, this criterion of 0.5% for local mortality is intended, as stated above, as an example only. The EFSA GMO Panel reiterates that the setting of criteria to define the need for specific mitigation measures is the task of the risk managers at the local level; any threshold applied must of necessity be arbitrary and should be subject to amendment according to local protection goals of each Member State.

Lepidopteran larvae are relatively sedentary, so any additional risk due to their potential movement towards *Bt*-maize fields is negligible. Furthermore, whilst lepidopteran adults are more mobile, and may potentially, for example through appetitive flight, travel the distance from such a protected habitat to a nearby *Bt*-maize field, they have very limited exposure to *Bt*-maize pollen. This is because, firstly, maize is, if at all, a host-plant to very few lepidopteran species of conservation concern (e.g., Schmitz et al., 2003). Also, most lepidopteran adults consume mainly nectar and plant sugars and the ability to feed on pollen has been described for a few species only. Relevant publications by Boggs (1987), Romeis et al. (2005) and Wäckers et al. (2007) discuss this in more detail. For these reasons, the environmental risk assessment of NT Lepidoptera for *Bt*-maize has focussed on larvae and not adults.

[17] See Table 2 and Section 3.1.5 on risk mitigation measures to reduce the exposure of non-target lepidopteran species of conservation concern and protected habitats to maize Bt11 pollen in EFSA (2011a).

3.1.2.4. The relationship between estimates of global and of local mortality

Within the mathematical model, estimated global mortality is related to estimated local mortality through the equation:

$$\text{Estimated global mortality} = R \text{ x Estimated local mortality}$$

where the product parameter *R* was defined above (see Section 3.1.2.1, above).

EFSA[18] (2011a) provides estimates of the five large-scale parameters and of their product, *R*. The EFSA GMO Panel used four cases: $R = 0.08$ ('conservative') ; $R = 0.02$ ('precautionary') ; $R = 0.0049$ ('typical') ; and $R = 0.00024$ ('non-conservative'). Here, we focus on the first three values.

As an example, for local mortality, the threshold value of 0.5%, mentioned in section 3.1.2.3 above, corresponds to a global mortality threshold no greater than 0.04% (less than 1 individual in every 2500), even under a conservative assumed value for *R* of 0.08. For a smaller value of *R*, as would be usual, global mortality would be considerably less.

3.1.2.5. Terminology related to the quantification of risk

In EFSA (2011a), the EFSA GMO Panel suggested a set of management options to mitigate the risk according to the principle of proportionality. The EFSA GMO Panel pointed out that conservative assessments were made based on worst-case assumptions on the sensitivity of NT lepidopteran species to the Cry1Ab protein. The recommended mitigation measures described in EFSA (2011a) were made in line with these worst-case assumptions. The EFSA GMO Panel recognises that the final decision as to whether any particular value of an endpoint, such as mortality, is of '*no concern*', is for risk managers rather than for risk assessors.

However, the EFSA GMO Panel acknowledges that terms such as '*negligible'* and '*of no concern*' should be quantified and defined when used in its scientific outputs (EFSA, 2010a). Furthermore, the EFSA Scientific Opinion on Risk Assessment Terminology states that: "*certain words such as "negligible", "concern" and "unlikely", have risk management connotation in everyday language. The Scientific Committee recommends that, when used in EFSA Scientific Opinions, they should be used carefully with objective scientific criteria (not involving value judgments) and be clearly defined so as to avoid the impression that risk assessors are making risk management judgments"* (EFSA, 2012d).

One of the main objectives of Directive 2001/18/EC is to protect human and animal health and the environment from risks associated with the deliberate release of GMOs into the environment (EC, 2001). The Directive therefore defines environmental protection goals in generic terms, referring to terms such as environment, biodiversity and NTOs, including NT Lepidoptera. In addition to these protection goals, the Commission Decision 2002/623/EC (EC, 2002) supplementing Annex II of Directive 2001/18/EC refers to the functioning of the ecosystem, and Directive 2004/35/EC (EC, 2004) on environmental liability defines any damage as representing a measurable adverse change in a natural resource/resource service.

Overall, aspects of the environment to be protected from harm can be divided into two discrete but inter-connected categories: (1) the protection of biodiversity (e.g., to maintain the favourable conservation status of a Lepidopteran species); and (2) the protection of the ecological and anthropocentric functions provided by ecosystem services[19]. Regarding (1), significant adverse changes to the baseline condition should be determined by means of measurable data (e.g., the number/density of lepidopteran individuals, their role in relation to species conservation, the rarity of

[18] See Section 2.2.3.5 in EFSA (2011a)

[19] Valued ecosystem services to preserve in an agricultural context are pest regulation, pollination, decomposition of organic matter, soil nutrient cycling, soil structure, water regulation and purification, and cultural services (such as aesthetic value).

the species, the species capacity for propagation and, after damage has occurred, to recover within a short time, without any intervention other than increased protection measures).

The implications of these aspects of population dynamics for environmental risk assessment were discussed by the EFSA GMO Panel in Section 2.2.2 of EFSA (2010a) and Section 1.5 of EFSA (2010b). In accordance with Annex 1 of Directive 2004/35/EC, the term '*significant damage*' is interpreted as excluding: (1) negative variations that are smaller than natural fluctuations regarded as normal for the species in question; (2) negative variations due to natural causes or resulting from intervention relating to the normal management of sites, including appropriate pesticide usage; and (3) damage to species for which it is established that they will recover, within a short time and without intervention, either to the baseline condition or to a condition which leads, solely by virtue of the dynamics of the species or habitat, to a condition deemed equivalent or superior to the baseline condition.

3.2. Specific supplementary data requested for mortality of non-target Lepidoptera

3.2.1. Question 1: Local mortality of non-target Lepidoptera where there is a strip of non-*Bt*-maize that is 21 m wide around the perimeter of the *Bt* crop and either a) 2 m field margin or b) no field margins

In EFSA (2011a), the EFSA GMO Panel provided estimates[20] of local and global percentage mortalities of NT lepidopteran larvae for a typical 15 ha square maize Bt11/MON 810 field with a 2 m margin on four sides and with no margins. The EFSA GMO Panel also accounted for none (i.e., no non-*Bt*-maize border rows) and full mitigation (i.e., non-*Bt*-maize border rows of 24 m width for a 15 ha field and pro-rata for fields of different sizes). Here, the EFSA GMO Panel presents supplementary data to calculate estimated local and, for completeness, global percentage mortalities for a similar field with and without margins[21] considering a 21 m wide strip of non-*Bt*-maize.

For such a field, strips of width 20.45 m would yield the required 20% non-*Bt*-maize in the assumed field, a percentage which is recommended at the farm level by North American Authorities and in EFSA (2011a) as non-*Bt*-*refugia* to delay the evolution of resistance to *Bt*-toxins amongst target pest species. Hence results for a 21 m wide strip (yielding 20.51% *refugia*) closely approximate to that which would pertain if the recommendations for IRM in EFSA (2011a) were implemented. Mortalities are calculated both when host-plants are absent within the crop ($e = 0.0$) and when the number of within-crop host-plants/m², e, is 0.01 (it is assumed throughout that the crop itself is not a host-plant for any of the NT lepidopteran species being considered).

Results are displayed in Table 1, below.

[20] See Table 1 in EFSA (2011a).

[21] See definition in Section 2.

Table 1: Estimates of local and global percentage mortality of NT lepidopteran larvae of different sensitivities, for a 15 ha maize Bt11 (equally applicable to maize MON 810) field with a 2 m margin or no margins, a within-crop host-plant density of $e = 0.01/m^2$ or no within-crop host-plants, for mitigation in the form of 21 m wide non-*Bt*-maize strips around the edges of the GM field.

	Mortality		Local mortality		Global mortality					
	R		1.0		0.08 'Conservative'		0.02 'Precautionary'		0.0049 'Typical'	
Sensitivity	Mitigation		None	21 m wide non-*Bt*-maize strips	None	21 m wide non-*Bt*-maize strips	None	21 m wide non-*Bt*-maize strips	None	21 m wide non-*Bt*-maize strips
	Host-plant density within the crop	Margin								
below average	zero	2 m	6.0×10^{-3}	$< 10^{-5}$	4.8×10^{-4}	$< 10^{-5}$	1.2×10^{-4}	$< 10^{-5}$	$< 10^{-5}$	$< 10^{-5}$
below average	0.01	2 m	0.0125	7.1×10^{-3}	1.0×10^{-3}	5.6×10^{-4}	2.5×10^{-4}	1.4×10^{-4}	6.1×10^{-5}	3.4×10^{-5}
below average	0.01	none	0.023	0.018	1.8×10^{-3}	1.4×10^{-3}	4.5×10^{-4}	3.6×10^{-4}	1.1×10^{-4}	8.8×10^{-5}
above average	zero	2 m	0.081	4.4×10^{-5}	6.5×10^{-3}	$< 10^{-5}$	1.6×10^{-3}	$< 10^{-5}$	4.0×10^{-4}	$< 10^{-5}$
above average	0.01	2 m	0.17	0.097	0.014	7.8×10^{-3}	3.4×10^{-3}	1.9×10^{-3}	8.3×10^{-4}	4.7×10^{-4}
above average	0.01	none	0.31	0.25	0.025	0.02	6.2×10^{-3}	5.0×10^{-3}	1.5×10^{-3}	1.2×10^{-3}
high	zero	2 m	1.1	5.9×10^{-4}	0.088	4.7×10^{-5}	0.022	1.1×10^{-5}	5.4×10^{-3}	$< 10^{-5}$
high	0.01	2 m	2.3	1.3	0.18	0.10	0.046	0.026	0.011	6.4×10^{-3}
high	0.01	none	4.2	3.3	0.33	0.26	0.083	0.066	0.020	0.016
I.io & *V.atalanta*	zero	2 m	2.1	1.2×10^{-3}	0.17	9.6×10^{-5}	0.043	2.4×10^{-5}	0.011	$< 10^{-5}$
I.io & *V.atalanta*	0.01	2 m	4.5	2.5	0.36	0.20	0.089	0.050	0.022	0.012
I.io & *V.atalanta*	0.01	none	8.1	6.5	0.64	0.52	0.16	0.13	0.040	0.032
P. xylostella	zero	2 m	3.5	2.0×10^{-3}	0.28	1.6×10^{-4}	0.070	4.0×10^{-5}	0.017	$< 10^{-5}$
P. xylostella	0.01	2 m	7.2	4.1	0.58	0.33	0.14	0.082	0.036	0.020
P. xylostella	0.01	none	13.1	10.5	1.05	0.84	0.27	0.21	0.064	0.051
very high	zero	2 m	13.0	8.1×10^{-3}	1.0	6.5×10^{-4}	0.26	1.6×10^{-4}	0.064	4.0×10^{-5}
very high	0.01	2 m	26.4	14.9	2.1	1.2	0.53	0.30	0.13	0.073

very high	0.01	none	47.2	38.1	3.8	3.0	0.94	0.76	0.23	0.19
extreme	zero	2 m	65.9	0.11	5.3	8.8×10^{-3}	1.4	2.2×10^{-3}	0.32	5.4×10^{-4}
extreme	0.01	2 m	79.2	32.6	6.3	2.6	1.6	0.65	0.39	0.16
extreme	0.01	none	100	83.3	8.0	6.7	2.0	1.7	0.49	0.41

The conclusion from the above results is that, within agricultural landscapes, when a field cropped with maize Bt11/MON 810 has no margins containing host-plants of NT lepidopteran larvae, the only larvae exposed are those on any host-plants within the crop. When such host-plants are present (i.e. here for $e = 0.01$), a greater percentage of the larvae exposed to *Bt*-maize pollen are expected to suffer mortality than in the corresponding situation[22] when a field has 2 m margins with host-plants. This is the case despite the fact that fewer individual larvae are expected to suffer mortality (because there are fewer individual larvae exposed).

The EFSA GMO Panel consider that these results have no implications concerning policy for the establishment or retention of field margins. Of course it is the case that when there are no host-plants within the crop ($e = 0.00$) expected mortality is less when there are no margins than when there are margins, but this must be set against the fact that in most agricultural situations field margins with host-plants contribute to the level of the regional population, as they may provide resources that are not available otherwise. The population in regions where most fields have margins therefore may well be greater than in regions where they do not, whatever the density of host-plants within the crop. The precise balance of the benefits of margins in this case is complex and depends on many factors, especially the parameter e and the parameter y (see Section 3.1.2.1, above), so it is difficult to draw clear conclusions. However, the EFSA GMO Panel emphasizes that field margins are a vital resource for the flora and fauna that comprise farmland biodiversity (Marshall et al., 2006; Pywell et al., 2012), and an important component of wildlife-friendly farming within Europe, supported through agri-environment schemes incorporated into the Common Agricultural Policy.

3.2.2. Question 2: The influence of non-*Bt-refugia* spatial arrangements on the local mortality of non-target Lepidoptera

3.2.2.1. Mitigation in the form of a single block of non-*Bt*-maize placed on one side of the *Bt*-maize field

EFSA (2011a) considered a 15 ha square maize Bt11/MON 810 field with mitigation in the form of strips of non-*Bt*-maize of width w arranged on each of the four sides of the field, ranging from $w = 0$ (no mitigation), through $w = 3, 6, 9, 12, 15, 18, 21$, to $w = 24$ m. In particular, it was noted above that results for a 21 m wide strip would yield 20.51% non-*Bt-refugia*, which closely approximate to that which would pertain if the recommendations for IRM in EFSA (2011a) were implemented[23].

Here, the EFSA GMO Panel presents supplementary data to calculate estimated local and, for completeness, global mortalities when either there is no mitigation or when the mitigation is in the form of a single block of non-*Bt*-maize on one side of the *Bt*-maize field (see Tables 2 and 3, below). To achieve a value of 20.51 % non-*Bt-refugia* (i.e., comparable with the 21 m wide strips), the block will be 79.4 m wide, as shown in Figure 1, below, with the block represented by black shading. The EFSA GMO Panel provided estimates of global mortalities which are calculated on the basis of three different values of the parameter R (see Section 3.1.2.4, above).

For conclusions, see Section 3.2.2.4, below.

[22] See Table 1 in EFSA (2011a).
[23] See Section 3.1.6 on conclusions on risk mitigation measures in EFSA (2011a).

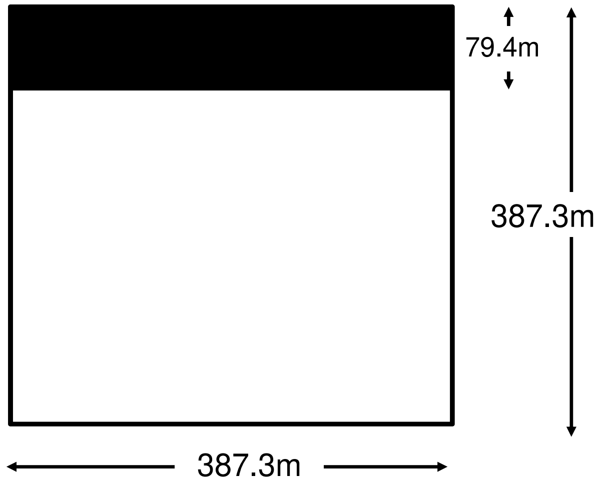

Figure 1: A 15 ha field, 20.51% of which consists of non-*Bt*-maize (shown shaded black) in the form of a single block with dimensions 79.4 m × 387.3 m placed on one side of the field. The remaining part of the field is cropped with *Bt*-maize (shown shaded white) in the form of a single block with dimensions 307.9 m × 387.3 m.

The calculations use exactly those combinations of the parameters of *R* and *e* adopted above in Section 3.2.1, and are repeated firstly for a field with a 2 m margin (Table 2, below) and then for a field with no margins (Table 3, below).

Table 2: Estimates of local and global percentage mortality of NT lepidopteran larvae of different sensitivities, for a 15 ha maize Bt11 (equally applicable to maize MON 810) field with a 2 m margin and within-crop host-plant density of *e* = 0.01/m², for mitigation in the form of a single block of non-*Bt*-maize of width 79.4 m placed on one side of the GM field.

Sensitivity	Mortality		Local mortality		Global mortality					
	R		1.0		0.08 'Conservative'		0.02 'Precautionary'		0.0049 'Typical'	
	Mitigation		None	Single block on side of field	None	Single block on side of field	None	Single block on side of field	None	Single block on side of field
	Host-plant density within the crop	Margin								
below average	0.01	2 m	0.0125	9.5×10^{-3}	1.0×10^{-3}	7.6×10^{-4}	2.5×10^{-4}	1.9×10^{-4}	6.1×10^{-5}	4.6×10^{-5}
above average	0.01	2 m	0.17	0.13	0.014	0.010	3.4×10^{-3}	2.6×10^{-3}	8.3×10^{-4}	6.3×10^{-4}
high	0.01	2 m	2.3	1.7	0.18	0.14	0.046	0.034	0.011	8.4×10^{-3}
Inachis io & *Vanessa atalanta*	0.01	2 m	4.5	3.4	0.36	0.27	0.089	0.067	0.022	0.016
Plutella xylostella	0.01	2 m	7.2	5.5	0.58	0.44	0.14	0.11	0.036	0.027
very high	0.01	2 m	26.4	19.9	2.1	1.6	0.53	0.40	0.13	0.098
extremely high	0.01	2 m	79.2	57.8	6.3	4.6	1.6	1.2	0.39	0.28

Table 3: Estimates of local and global percentage mortality of NT lepidopteran larvae of different sensitivities, for a 15 ha maize Bt11 (equally applicable to maize MON 810) field with no margins and within-crop host-plant density of $e = 0.01/m^2$, for mitigation in the form of a single block of non-*Bt*-maize of width 79.4 m placed on one side of the GM field.

Sensitivity	Mortality ▸		Local mortality		Global mortality					
	R ▸		1.0		0.08 ‘Conservative’		0.02 ‘Precautionary’		0.0049 ‘Typical’	
	Mitigation ▸		None	Single block on side of field	None	Single block on side of field	None	Single block on side of field	None	Single block on side of field
	Host-plant density within the crop	Margin								
below average	0.01	none	0.023	0.018	1.8×10^{-3}	1.4×10^{-3}	4.5×10^{-4}	3.6×10^{-4}	1.1×10^{-4}	8.9×10^{-5}
above average	0.01	none	0.31	0.25	0.025	0.020	6.2×10^{-3}	4.9×10^{-3}	1.5×10^{-3}	1.2×10^{-3}
high	0.01	none	4.2	3.3	0.33	0.26	0.083	0.066	0.020	0.016
Inachis io & *Vanessa atalanta*	0.01	none	8.1	6.4	0.64	0.51	0.16	0.13	0.040	0.031
Plutella xylostella	0.01	none	13.1	10.4	1.05	0.84	0.27	0.21	0.064	0.051
very high	0.01	none	47.2	37.7	3.8	3.01	0.94	0.75	0.23	0.18
extremely high	0.01	none	100	80.5	8.0	6.44	2.0	1.6	0.49	0.39

3.2.2.2. Mitigation in the form of a single block of non-*Bt*-maize placed remotely outside the *Bt*-maize field

Here, the EFSA GMO Panel presents supplementary data to calculate estimated local and global mortalities when either there is no mitigation or when the mitigation is in the form of a single block of non-*Bt*-maize placed remotely outside the *Bt*-maize field. To achieve a value of 20.51% *refugia* (i.e., comparable with the 21 m wide strips), the remote block will be 99. 9 m wide, as shown in Figure 2, below, with the block represented by black shading.

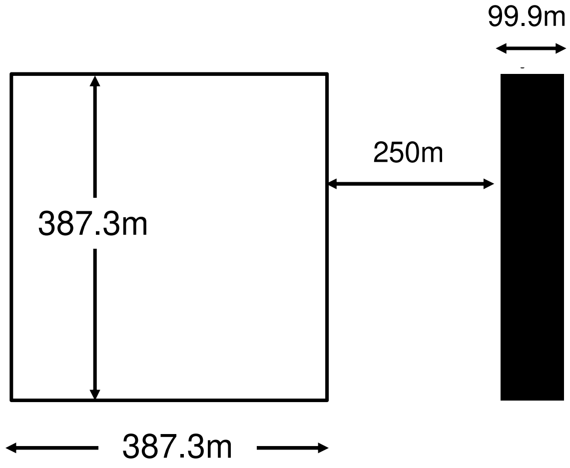

Figure 2: A square 15 ha field, cropped entirely with *Bt*-maize (shown shaded white) and a single block of non-*Bt*-maize with dimensions 99.9 m × 387.3 m (shown shaded black) placed remotely, 250 m from the field (example distance only, since precise distance from field not critical within a certain range). The total area of all the maize planted is 18.69 ha, of which 20.51% comprises non-*Bt*-maize.

As above, the calculations use exactly those combinations of the parameters of *R* and *e* adopted in Sections 3.2.1 and 3.2.2, and are repeated firstly for a field with a 2 m margin (Table 4, below) and then for a field with no margins (Table 5, below).

For conclusions, see Section 3.2.2.4, below.

Table 4: Estimates of local and global percentage mortality of NT lepidopteran larvae of different sensitivities, for a 15 ha maize Bt11 (equally applicable to maize MON 810) field with a 2 m margin and within-crop host-plant density of $e = 0.01/m^2$, for mitigation in the form of a single block of non-*Bt*-maize with dimensions 99.9 m x 387.3 m, placed remotely outside the field.

Sensitivity	Mortality		Local mortality		Global mortality					
	R		1.0		0.08 'Conservative'		0.02 'Precautionary'		0.0049 'Typical'	
	Mitigation		None	Single block placed remotely	None	Single block placed remotely	None	Single block placed remotely	None	Single block placed remotely
	Host-plant density within the crop	Margin								
below average	0.01	2 m	0.0125	9.9×10^{-3}	1.0×10^{-3}	7.9×10^{-4}	2.5×10^{-4}	2.0×10^{-4}	6.1×10^{-5}	4.9×10^{-5}
above average	0.01	2 m	0.17	0.14	0.014	0.011	3.4×10^{-3}	2.7×10^{-3}	8.3×10^{-4}	6.6×10^{-4}
high	0.01	2 m	2.3	1.8	0.18	0.15	0.046	0.037	0.011	9.0×10^{-3}
Inachis io & *Vanessa atalanta*	0.01	2 m	4.5	3.6	0.36	0.29	0.089	0.072	0.022	0.018
Plutella xylostella	0.01	2 m	7.2	5.7	0.58	0.46	0.14	0.11	0.036	0.028
very high	0.01	2 m	26.4	21.0	2.1	1.68	0.53	0.42	0.13	0.10
extremely high	0.01	2 m	79.2	63.0	6.3	5.04	1.6	1.26	0.39	0.31

Table 5: Estimates of local and global percentage mortality of NT lepidopteran larvae of different sensitivities, for a 15 ha maize Bt11 (equally applicable to maize MON 810) field with no margins and within-crop host-plant density of $e = 0.01/m^2$, for mitigation in the form of a single block of non-*Bt*-maize with dimensions 99.9 m x 387.3 m, placed remotely outside the field.

Sensitivity	Mortality		Local mortality		Global mortality					
	R		1.0		0.08 ‘Conservative’		0.02 ‘Precautionary’		0.0049 ‘Typical’	
	Mitigation		None	Single block placed remotely	None	Single block placed remotely	None	Single block placed remotely	None	Single block placed remotely
	Host-plant density within the crop	Margin								
below average	0.01	none	0.023	0.018	1.8×10^{-3}	1.5×10^{-3}	4.5×10^{-4}	3.7×10^{-4}	1.1×10^{-4}	9.0×10^{-5}
above average	0.01	none	0.31	0.25	0.025	0.020	6.2×10^{-3}	4.9×10^{-3}	1.5×10^{-3}	1.2×10^{-3}
high	0.01	none	4.2	3.3	0.33	0.27	0.083	0.065	0.020	0.016
Inachis io & *Vanessa atalanta*	0.01	none	8.1	6.4	0.64	0.52	0.16	0.13	0.040	0.032
Plutella xylostella	0.01	none	13.1	10.4	1.05	0.83	0.27	0.21	0.064	0.051
very high	0.01	none	47.2	37.5	3.8	3.0	0.94	0.75	0.23	0.18
extremely high	0.01	none	100	79.4	8.0	6.4	2.0	1.6	0.49	0.39

3.2.2.3. Mitigation in the form of alternative spatial arrangements

In general, when a *Bt*-maize field has margins with host-plants of NT Lepidoptera, strips of non-*Bt*-maize planted between the *Bt*-maize and the margins provide the most effective mitigation measure for reducing exposure to *Bt*-maize pollen and hence the mortality of NT lepidopteran larvae . Of course, other forms of spatial arrangements of mitigation are possible, but these must be decided on a case-by-case basis. For example, if there were only a single margin containing host-plants on one side of a square field then a single strip of non-*Bt*-maize placed between the *Bt*-maize and this margin would be the most effective mitigation tool. Similarly, if there were two margins containing host-plants, then there would need to be two strips alongside each of the margins (see Figure 3, below, for an illustration of this situation). The dimensions of the strips will need to be calculated to give the required percentage *refugia* (i.e. to yield a total 20% of the total area cropped with *Bt*-maize at the farm level) depending on the number and location of non-*Bt*-maize strips and the amount of *Bt*-maize.

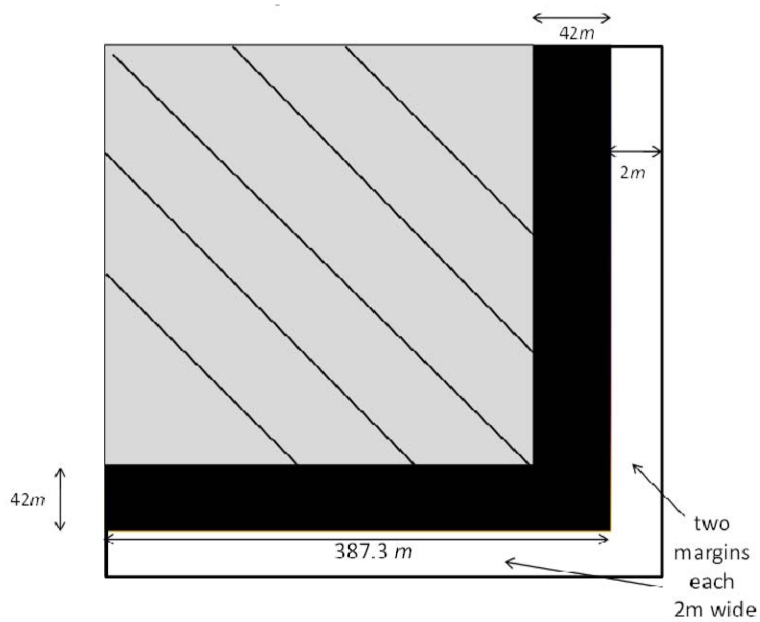

Figure 3: Illustrative example of a non-standard arrangement of margins, strips of non-*Bt*-maize and of *Bt*-maize, where dimensions require careful calculation on a case-by-case basis. The figure is not in scale; black shaded area indicates non-*Bt*-maize; 20.51% of total field area; grey shaded area with hatching indicates *Bt*-maize; field margins shown in white.

3.2.2.4. Conclusions regarding the influence of size, shape and location of non-*Bt*-maize *refugia* on local and global mortality of non-target Lepidoptera

The EFSA GMO Panel draws the following conclusions from the results presented in Tables 2-5, above. In Table 2, where the field has margins, the strips are considerably more effective at reducing mortality than the single block of non-*Bt*-maize planted on one side of the field with comparable *refugia* area. This is the case whether there are host-plants in the crop or not. For Table 3, where the field has no margins and there are some host-plants within the crop, slightly fewer (between 0% and 2.8% depending on the sensitivity category) larvae are expected to suffer mortality when there is a single block of non-*Bt*-maize planted on one side of the field than when there are strips along each side with comparable *refugia* area. For Table 4, where the field has margins, the remotely-placed block is even less effective at reducing mortality than the single block placed on one side of the field. Hence, again, the strips are very considerably more effective (always more than 40%, often at least twice as effective, depending on the sensitivity category) at reducing mortality than the single block of non-*Bt*-

maize planted remotely outside the field with comparable *refugia* area. This is the case whether there are host-plants in the crop or not. For Table 5, where the field has no margins and there are some host-plants within the crop, slightly fewer (between 0% and 3.9% depending on the sensitivity category) larvae are expected to suffer mortality when the single block of non-*Bt*-maize is planted remotely, outside the field, than when it is planted on one side of the field (and, as above, than when there are strips along each side) for blocks and strips with comparable *refugia* area.

The EFSA GMO Panel reiterates that, in general, for NT Lepidoptera, spatial arrangements of non-*Bt*-maize should seek to maximise the average distance of larvae from the nearest source of maize Bt11/MON 810 pollen, since these minimise exposure (EFSA, 2011a). The overall conclusion from the results above is that when a *Bt*-maize field has margins with host-plants of NT Lepidoptera, then strips of non-*Bt*-maize planted between the *Bt*-maize and the margins provide the most effective mitigation in reducing the mortality of NT lepidopteran larvae from the exposure to *Bt*-maize pollen. This is the case however many margins there are (i.e., one, two, three or four).

Perry (2011b) also studied the optimal size and placing of blocks of non-*Bt*-maize. His conclusions were consistent with those above. Specifically, Perry (2011b) drew attention to the use of *Bt*-/non-*Bt*-maize seed mixtures for IRM and integrated pest management in North America. Assuming a thorough mixing of the *Bt*- and non-*Bt*-seed, seed mixtures would tend to minimise, rather than maximise, the average distance of larvae from the nearest source of *Bt*-maize pollen. Hence, regardless of their efficacy for IRM, seed mixtures provide the poorest possible efficacy of mitigation to limit the exposure of NT Lepidoptera to *Bt*-maize pollen (EFSA, 2012e).

3.2.3. Question 3: Local mortality of non-target lepidopteran species with increasing distances from the nearest maize Bt11/MON 810 field and where there are no field margins

It is necessary to issue some clarification regarding the calculations underlying the isolation distances quoted in Table 2 of EFSA (2011a). The calculations given there related to a field with no margins.

The calculation of local mortality, g(E), at different distances, E, from the crop is derived from equation 2 of the Perry et al. (2012):

$$g(E) = \exp(-0.35853E)/[\beta + \exp(-0.35853E)]$$

where the values of β for different sensitivities are, respectively:

- β = 0.003893, extremely high;
- β = 0.05290, very high;
- β = 0.7190, high;
- β = 9.774, above-average; and
- β = 132.9, below-average.

As given in Table 2 of EFSA (2011a), for a field with no margins, these formulae give the values shown in Table 6, below.

Table 6: The dependence of estimated local mortality (%) of a range of hypothetical NT lepidopteran larvae of differing sensitivities, including *Plutella xylostella* and *Inachis io/Vanessa atalanta*, on increasing distances from the nearest field (assuming no margins) cropped with maize Bt11 (equally applicable to maize MON 810)

Distance from *Bt*-maize field (m)	**Sensitivity**						
	Below average	Above average'	High	*I. io / V. atalanta*	*P. xylostella*	Very high	Extremely high
2	0.0	0.1	0.7	1.4	2.4	9.2	57.8
5	0.0	0.0	0.3	0.5	0.8	3.3	31.8
10	0.0	0.0	0.0	0.1	0.1	0.6	7.2
15	0.0	0.0	0.0	0.0	0.0	0.1	1.3
20	0.0	0.0	0.0	0.0	0.0	0.0	0.2
25	0.0	0.0	0.0	0.0	0.0	0.0	0.0

For a field with a 2 m margin, an individual larva that is distance E from the field is actually at a distance of $E + 2$ from the *Bt*-crop. Then the following values are obtained (see Table 7, below):

Table 7: The dependence of estimated local mortality (%) of a range of hypothetical NT lepidopteran larvae of differing sensitivities, including *Plutella xylostella* and *Inachis io/Vanessa atalanta*, on increasing distances from the nearest field (assuming a 2 m margin) cropped with maize Bt11 (equally applicable to maize MON 810)

Distance from *Bt*-maize field (m)	**Sensitivity**						
	Below average	Above average'	High	*I. io / V. atalanta*	*P. xylostella*	Very high	Extremely high
2	0.0	0.1	0.4	0.7	1.2	4.7	40.1
5	0.0	0.0	0.1	0.2	0.4	1.7	18.6
10	0.0	0.0	0.0	0.0	0.1	0.3	3.7
15	0.0	0.0	0.0	0.0	0.0	0.0	0.6
20	0.0	0.0	0.0	0.0	0.0	0.0	0.1
25	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 2 of EFSA (2011a) may therefore be seen to be conservative, in the sense that the expected mortalities quoted there for a field without margins are greater than those above, where the field does have a 2 m margin.

Concerning the implications for recommendations for mitigation, EFSA (2011a)[24] stated that: "*however, the EFSA GMO Panel considers that lepidopteran species of conservation concern with unknown sensitivity to the Cry1Ab protein occurring in protected habitats according to Directive 2004/35/EC require additional protection and, in these cases recommend that maize Bt11 is not cultivated within 20 m of the boundary of these habitats, so that exposure and hence the risks to larvae of these lepidopteran populations are minimised in these habitats*".

[24] See Section 3.1.6 on conclusions on risk mitigation measures in EFSA (2011a).

Since the greatest mortality occurs for the case of a field with no margins, and since even for such a field the mitigation recommendation of a isolation distance of 20 m would be expected to reduce the local mortality to a level at or below 0.5%, the recommendation can be seen to be conservative and to hold even for worst-case scenarios. The conclusion is that the recommended isolation distance in EFSA (2011a) is valid for all fields of maize Bt11/MON 810.

3.2.4. Question 4: The influence of local and regional conditions on Insect Resistance Management (IRM) plan

3.2.4.1. Regionally occurring non-target lepidopteran pests of maize in the EU

Aspects of Environmental Risk Assessment

In addition to the relatively widespread target pests as defined by the applicant (i.e., ECB and MCB), there are other lepidopteran species (*S. cretica*, *Helicoverpa armigera*, *Mythimna unipuncta*, *Agrotis segetum*, *A. ipsilon, Autographa gamma*), that may occur and cause damage to maize in some years in certain EU regions (Meissle et al., 2010, 2012; CABI Invasive Species Compendium, 2012; EPPO Global Database, 2012). These species are either not widespread over the entire European maize growing area (e.g., *H. armigera*), or do not regularly occur at high population densities (e.g., *S. cretica*) or cause damage to maize only in certain years (e.g., *A. segetum* and *H. armigera*) due to factors such as: their biology (outbreak cycles referred to by Mészáros and Nagy, 1968), temperature requirements (Balogh et al., 2008), or migratory behaviour (Vojnits, 1966). The implications for exposure are explored below.

Many of these species demonstrate an outbreak cycle, often of 4-8 years (Mészáros and Nagy, 1968; Balogh et al., 2008). The occurrence and density of these pests are difficult to forecast, therefore they are usually not part of routine pest management schemes by farmers which plan specific insecticide treatments in advance of any expected infestations. Some of these species (*H. armigera, A. gamma, M. unipuncta*) migrate towards EU member states and the majority of their populations are not able to overwinter in the EU regions concerned where their larvae feed on maize (e.g., for *H. armigera* see Lammers and MacLeod (2007)). Therefore, the potential for evolving resistance in these species might be lower compared to species which normally overwinter in EU regions such as ECB and MCB, because their larvae are not exposed frequently to plant-produced Cry1Ab protein. In addition, in some species such as *H. armigera,* large-scale migration of populations allows high gene flow (Feng et al., 2005) that facilitates the exchange of genetic material between exposed and unexposed populations, which therefore would tend to delay the evolution of resistance.

In addition, all above listed lepidopteran species (and in general other noctuid lepidopteran pests) are highly polyphagous, their larvae feeding on cultivated crops (i.e., cereals, root crops, vegetables), fruit trees, ornamentals, broad leaf weeds, grasses and wild plants (Koch, 1984; CABI Invasive Species Compendium, 2012; EPPO Global Database, 2012). Therefore, in addition to maize, the larval populations of these species may feed and develop on a broad range of host-plants available in agricultural landscapes.

Exposure is also related to the degree of multivoltinity of the species concerned. Larvae of certain species (*H. armigera, A. gamma*) feed almost exclusively on generative plant parts (Lammers and MacLeod, 2007). The egg laying and feeding of larvae of *H. armigera* on maize is in strong synchrony with the maize crop phenology; egg laying starts at the R1 (silking) stage of maize (Dömötör et al., 2007, 2009), so only one larval generation of the three typical for the Pannonian biogeographical region will develop on maize. Therefore, neither the previous nor the subsequent generation of this species will be exposed to the Cry1Ab protein, reducing still further the exposure of the overall population.

For all the reasons detailed above, it is expected that a component of the population of each of these lepidopteran species would not be exposed to Cry1Ab protein from maize Bt11/MON 810. The size of this unexposed component of the population will depend on local and regional conditions (e.g., the availability of host-plants and habitats, the dispersal behaviour of the species, its spatial pattern at the landscape scale, etc), so cannot easily be estimated.

Based on the details discussed above concerning host-plants, biology, migratory behaviour, and the sporadic occurrence of these regionally occurring pests, the spatial and temporal exposure of their larvae to Cry1Ab protein is likely to be considerably less than that of target pests and lead to reduced selection pressure on these species. Therefore, routine IRM would not be proportionate.

Aspects of Risk Management

Potential *Bt*-maize hybrids are selected for commercialisation on the basis that the *Bt*-toxin itself and its expression levels have been optimized to ensure appropriate efficacy against certain target pests. These hybrids may have various efficacy levels against other lepidopteran pests (e.g., the regionally occurring ones listed above). However, it is known from the literature (e.g., Eizaguirre et al., 2006; Erasmus et al., 2010) and from technical documents submitted by the applicant, that maize MON 810 hybrids (expressing the Cry1Ab protein) have little or no effect on cutworm (*A. segetum*) or on infestation levels of *H. armigera* larvae compared to non-*Bt*-maize in Spain (Eizaguirre et al., 2010). In contrast, Kiss et al. (2003) observed decrease of *H. armigera* larval density in Hungary in a similar comparison. Notwithstanding, a medium or even a low efficacy dose may decrease the population level of these pests below economic threshold levels (depending on their initial population density), and in this instance the efficacy and control level achieved would be in line with Integrated Pest Management (IPM) principles (Meissle et al., 2011).

An efficient IRM strategy against a particular target species mostly relies on the 'high dose/refuge' strategy[25]. One underlying assumption of the strategy is that the *Bt*-maize must produce a very high concentration of the *Bt*-toxin (25 times the amount needed to kill > 99% of susceptible individuals), so that nearly all target insects that are heterozygous for the resistance allele do not survive. As described above, there are cases where other *Bt*-toxins (including Cry1Ab) show efficacy levels well below 99%, against some regionally occurring non-target lepidopteran pests. It is for this reason that the EFSA GMO Panel pointed out, in EFSA (2011a), that for some of these regionally occurring non-target lepidopteran pest species, the Cry1Ab protein might not be expressed in relevant plant tissues at a sufficiently toxic dose to fulfil the conditions of the 'high dose/refuge' strategy. This might thwart efforts to delay resistance evolution to maize Bt11/MON 810 for these species.

The EFSA GMO Panel considers that, since the larvae of these other regionally occurring non-target lepidopteran pests will be exposed to lepidopteran active *Bt*-toxin(s) through their feeding on maize plants, they have the potential to evolve resistance to these toxins. Additionally, the EFSA GMO Panel wishes to stress that it is very difficult to assess the likely efficacy of the maize Bt11/MON 810 high dose/refuge strategy (as developed for the target pests) for the above mentioned regionally occurring non-target lepidopteran pests. For an assessment of whether this refuge strategy will work on these other species, additional information on all underlying assumptions of the strategy would be needed. It is possible that exploitation of *Bt*-maize to control these regionally occurring non-target lepidopteran pests may happen in some regions. However, for the reasons stated above, it is not considered proportionate to develop routine IRM for these regionally occurring non-target lepidopteran pests.

The EFSA GMO Panel advises that the potential of resistance evolution should be considered within Post-Market Environmental Monitoring (PMEM) and that General surveillance (GS) shall be used to report information on unexpected larval damage to maize and observations on the occurrence and unexpected survival of lepidopteran larvae on *Bt*-maize plants. This data can be derived from the farmer questionnaires and also reports from plant inspection services[26] and other surveys of pest incidence in maize. This information can then be used to trigger subsequent investigations, including case-specific monitoring (CSM), if necessary. It may prove useful to focus sampling on areas of high

[25] See Section 3.1.3 on risk mitigation measures to delay resistance evolution to the Cry1Ab protein in maize Bt11 (2011a).

[26] For example, through the Pest Monitoring Systems established under Directive 2009/128/EC establishing a framework for Community action to achieve the sustainable use of pesticides (EC, 2009).

adoption rate of *Bt* maize and where these pests are known to occur. The applicants should take all these issues into account and amend their PMEM plans accordingly.

3.2.4.2. Adaptation of Insect Resistance Management plan to local and regional conditions

Tyutyunov et al. (2008) and, in particular, MacIntosh (2009) give very useful specific advice to risk managers concerning how IRM plans can be adapted appropriately according to local and regional conditions. Section 3.1.3 of EFSA (2011a) gave some specific recommendations, but also presents a broad context for IRM implementation which allows for future possible scenarios such as the presence of several *Bt*-transformation events in maize and in other crops, and the pyramiding of several *Bt*-toxins in the same GM plant (EFSA, 2012b).

As a first step in the adaptation of plans to local and regional conditions, it is clearly important to identify the characteristics of the target lepidopteran pests within a specific region. This identification should be done on the basis of the experience of local farmers and consultants, and by using relevant information from the scientific and extension agriculture literature. The potential source of pest adults developing from larvae not exposed to *Bt*-toxin and the random mating of these with those exposed to *Bt*-toxins will differ within and across regions. Therefore, the polyphagous or oligophagous character of these pests and the availability, location and distribution of food-plants (other than maize) of these pests within and between fields should be taken into account in each region. Other important factors that may vary within and across regions such as insecticide use and crop rotations should also be considered (Head and Greenplate, 2012).

Cropping systems, the implementation of IPM programmes within which the *Bt*-crop may be embedded and management practices may vary between regions (and possibly also within a region) and between intensive agriculture and less-intensive systems. Here, 'intensive' indicates agriculture that seeks high production levels through the utilization of crop monocultures and large fields with few semi-natural habitats or uncultivated areas; and 'less-intensive' indicates systems with a wider diversity of crops on smaller plots, or crops interspersed and mixed with uncropped areas within the landscape.

The risk of resistance evolution in target pests is impacted by these patterns, with increased risk associated with monoculture cropping and higher adoption of *Bt*-crops. Non-*Bt*-*refugia* recommended for IRM of the target pests may need to be adapted in size and location according to the species and the regional cultivation characteristics. Farmers should be informed through the stewardship programs for *Bt*-maize, of the options for managing resistance evolution in these pests so that *refugia* can be developed according to local requirements. For further details see Tyutyunov et al. (2008) and MacIntosh (2009).

3.3. Overall Conclusions and Recommendations

The environmental risk assessment of NT Lepidoptera for *Bt*-crops focuses on larvae, and not adults, feeding on *Bt*-maize pollen deposited on their host-plants.

Depending on the level of exposure to *Bt*-maize pollen, there is a potential hazard to NT lepidopteran larvae on their host-plants in fields cropped with non-Lepidoptera-active crops when they neighbour the maize Bt11/MON 810 field under consideration. However, the need for risk management should consider the distance from the nearest source of *Bt*-maize pollen and hence their exposure as well as the pest status of the species concerned.

Within agricultural landscapes, when a field cropped with maize Bt11/MON 810 has no margins containing host-plants of NT lepidopteran larvae, the only larvae exposed are those on any host-plants within the GM crop. When such host-plants are present, a greater percentage of the larvae exposed to *Bt*-maize pollen are expected to suffer mortality than when a field has margins with host-plants. This is the case despite the fact that fewer individual larvae are expected to suffer mortality (because there are fewer individual larvae exposed).

If a maize Bt11/MON 810 field has margins, then sown strips of non-*Bt*-maize, placed between the edges of the *Bt*-maize crop and each margin, are considerably more effective as a mitigation measure at reducing expected mortality than a single block of non-*Bt*-maize of comparable area, wherever the latter is planted. This is the case whether there are host-plants in the crop or not. By contrast, when a maize Bt11/MON 810 field has no margins, then a single block of non-*Bt*-maize is slightly more effective at reducing larval mortality than sown strips.

For NT lepidopteran species of conservation concern occurring within protected habitats, it is appropriate for thresholds used to derive recommendations for risk management to be based on a criterion of local mortality; whereas for NT lepidopteran larvae occurring within maize fields and their margins a criterion of global mortality is considered appropriate.

Seed mixtures (e.g., *refugia* in a bag) provide the poorest possible efficacy of mitigation because they do little to limit the exposure of non-target Lepidoptera to *Bt*-maize pollen.

In general, for any particular *Bt*-maize plant expressing Cry1 protein, the required isolation distance around protected habitats within which maize Bt11/MON 810 should not be cultivated increases with both the sensitivity of the NT lepidopteran larvae and the expression levels of the Cry1-protein in *Bt*-maize pollen. In the present case of maize Bt11/MON 810, it is confirmed that imposing an isolation distance of 20 m around a protected habitat from the nearest crop of maize Bt11/MON 810 would be expected to reduce local mortality even of extremely highly sensitive non-target lepidopteran larvae to a level below 0.5% (and that for this distance the expected local mortality would actually be 0.2% or lower). This estimated isolation distance is conservative, since it assumes high levels of sensitivity in NT lepidopteran larvae, and because larvae within the habitat will be at greater distances from the *Bt*-maize crop than those on the edge of the habitat.

The EFSA GMO Panel considers that the potential of regionally occurring non-target lepidopteran pests to evolve resistance exists but is considerably less than that of target pests and therefore routine IRM would not be proportionate. Hence, the EFSA GMO Panel reiterates that regionally occurring non-target lepidopteran pests should be considered within the PMEM. General surveillance shall be used to report information on unexpected larval damage to maize and observations on the occurrence and survival of lepidopteran larvae on *Bt*-maize plants. In addition, monitoring reports from regional plant inspection services should be used to trigger subsequent investigations, including CSM if necessary. The applicants should amend their PMEM plans accordingly.

This Scientific Opinion provides background scientific information to inform the decision-making processes; the EFSA GMO Panel reiterates that risk managers should choose risk mitigation and management measures that are proportionate to the level of identified risk according to the protection goals pertaining to their regions.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the European Commission, dated 13 September 2012, to the EFSA Executive Director requesting additional evidence to support previous EFSA opinions on maize Bt11.

2. Acknowledgement letter, dated 27 September 2012, from the EFSA Executive Director to the European Commission.

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